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13. ABSTRACT *(Maximum 200 Words)*

The Breast Cancer Training Program for Summer Undergraduates (BCTP-SU) has been established within the Eppley Cancer Research Institute of the University of Nebraska Medical Center (UNMC). The purpose of the BCTP-SU is to expand the Eppley's summer research program to add five additional undergraduates to train specifically in breast cancer research. Trainees participated in didactic and academic activities, including: 1) a 10 week, lab-intensive research project, mentored by one of the participating faculty; 2) a weekly seminar series in various aspects of cancer research, including breast cancer projects; and 3) a poster session and research forum at the end of the summer to highlight their research accomplishments. In the first year of the BCTP-SU, five outstanding students were recruited to the Eppley Institute. Three students helped develop new tools for breast cancer research (microarrays, improved gene knockout reagents, telomerase vectors) and the other two students evaluated genes implicated in breast cancer and growth control. Several poster presentations and two published abstracts have already resulted. All five students are continuing on in research and/or medical fields, consistent with the goals of the BCTP-SU. All five BCTP-SU students were women, and one was also a member of an under-represented minority.

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Breast Cancer Training Program for Summer Undergraduates
Kenneth H. Cowan, M.D., Ph.D., Principal Investigator
Annual Summary Report, Year 1: May 15, 2001-May 14, 2002

I. Introduction

The Breast Cancer Training Program for Summer Undergraduates (BCTP-SU) has been established within the Eppley Cancer Research Institute of the University of Nebraska Medical Center (UNMC). The purpose of the BCTP-SU is to expand the Eppley's summer undergraduate research program to add five additional students who will train specifically in breast cancer research. Trainees participate in didactic and academic activities including: 1) a 10 week, lab-intensive research project, mentored by one of the participating faculty; 2) a weekly seminar series in various aspects of cancer research, including breast cancer projects; and 3) a poster session and research forum at the end of the summer to highlight their research accomplishments. Recruitment of women and other under-represented minorities is a significant part of the undergraduate training.

II. Body

A. Student participants, their research projects, and their mentors

Five students for the BCTP-SU, plus six other students, were recruited from a pool of approximately 55 applicants. Thus the program is highly selective, admitting about 20% of the applicants. Students were selected based on their academic training, their statement of interest in cancer research, and letters of recommendation. Lab assignments were based in part on student preferences, from a list of available faculty mentors. All BCTP-SU students were assigned to training faculty identified in the original BCTP-SU application. Students arrived at UNMC on or about June 3 and stayed through August 10, for a total of 10 weeks. Table 1 shows the BCTP-SU student names, their undergraduate affiliations, their faculty mentors, and the title of their research projects.

Table 1. BCTP-SU students for reporting year

Student	Affiliation	Mentor	Title of research project
Janelle Kime	Luther College (IA)	Hollingsworth	Utilization of cDNA Microarrays to Investigate the Role of the MUC1 Tandem Repeat in Gene Expression
Carmen Garst	Morningside College (IA)	Ouellette	The Design and Characterization of Retroviral Vectors Expressing Human Telomerase RNA
Sadie West	University of Wyoming	Wagner	*In Vitro* Gene Deletion Using a Ligand-Inducible Cre Recombinase Expressed by a Newly-Developed Retroviral Vector.
Karen Ortiz Cruz	University of Puerto Rico - Cayey	Lewis	Generation of $KSR^{-/-}$ Mice in Multiple Genetic Backgrounds
Meagan Govig	St. Olaf College (MN)	Shull	Genetic Control of Growth in the ACI and Copenhagen Rat Srains

B. Weekly seminars and other educational offerings

A mandatory weekly seminar was held to help educate the students about key areas in cancer research. Specific research results from the speakers' laboratories were used to illustrate concepts and to demonstrate how research is used to extend our knowledge about cancer. Two meetings were utilized for panel discussions with faculty and students regarding graduate research opportunities. The dates, speakers, and seminar titles are shown in Table 2.

Table 2. Mandatory seminars

Date	Speaker	Title
6/7/01	Robert Lahue, Ph. D. Elli Rogan, Ph. D.	Orientation and Safety
6/14/01	Surinder Batra, Ph.D.	Mucin Expression in Pancreatic Adenocarcinoma
6/21/01	Elliott Bedows, Ph. D.	The Biochemical Side of the Bioinformatics Problem
6/28/01	James Shull, Ph. D.	Cancer Genetics
7/5/01	Michel Ouellette, Ph. D.	Telomeres and Senescence
7/12/01	Joyce Solheim, Ph. D.	The Basics of Immunology
7/19/01	Eppley Faculty Panel	Graduate Research Opportunities
7/26/01	Eppley Graduate Students	Life in Graduate School
8/2/01	Robert Lahue, Ph. D.	Wrap-up meeting and invitation to apply to Eppley Graduate Program
8/9/01	Summer students	Poster Day

In addition, students were encouraged to attend other campus seminars related to cancer research. These seminars were presented by visiting speakers, by Eppley Institute and UNMC faculty, and by Eppley graduate students. The list is shown in Table 3.

Table 3. Suggested seminars

Date	Speaker	Title
6/7/01	Kuan-The Jeang, M.D. Ph.D., Laboratory of Molecular Microbiology, NIAID, NIH	HTLV-I Transactivator/Transforming Protein Tax and dysregulation of a Mitotic Checkpoint for Aneuploidy
6/8/01	Robert Lewis, Ph.D. Breast Cancer Training Program, UNMC	Cell Models for Breast Cancer
6/8/01	Megan Sykes M.D., Professor of Surgery/Immunology, Massachusettes General/ Harvard	Non-Myeloblative Transplantation for Malignant and Non-Malignant Diseases
6/13/01	Julie M Vose, M.D., Professor, Oncology/Hematology UNMC/Eppley Cancer Center Grand Rounds	Immunotherapy for Non-Hodgkin's Lymphoma
6/14/01	Anne Kessinger, M.D. UNMC	Stem Cells and the Future of Medicine
6/21/01	Kim McDermott- Eppley Ph. D. student dissertation	Structure-Function Studies of Tumor-Associated MUC1: Primary Sequence of MUC1 Involved in Its Post-Translational Processing and Function
6/26/01	Robert Lahue Ph.D., Eppley Institute, UNMC	What's New with Triplet Repeat Instability?
7/25/01	Dr. Ralf Kuppers, UNMC	The Role of the Germinal center Reaction in B-cell Lymphomagenesis
7/31/01	Dr. Bhavana Dave, UNMC	Cytogenic Approches to Lyphoma Research
8/3/01	Lance Johnson - Eppley Ph. D. student dissertation	Transcription Factors Involved in the Regulation of the FGF-4 Gene

C. Poster presentations

Each student presented a poster on her research at the UNMC summer student research forum, held Aug. 9. The student author, mentor, and title of each poster are shown in Table 1. Copies of the poster title, authors, and abstract are presented in the Appendix of this report. These presentations are also referred to in section IV, Reportable Outcomes.

D. Recruitment of women and other under-represented minorities

All five BCTP-SU trainees in the reporting year were female. One student (Ortiz) is also a member of an under-represented minority.

E. Tracking of trainees

The current status of each BCTP-SU trainee is shown in Table 4. Three of the students (West, Ortiz, and Govig) have graduated from college. The other two students (Kime and Garst) will be seniors in the fall semester, 2002.

Table 4. Current status of trainees

Student	Educational Status	Awards, Honors, Current Situation
Sadie West	Graduated	Cum Laude, Phi Beta Kappa, Phi Kappa Phi. Received the Arts and Sciences Outstanding Graduate Award, and the Department of Zoology Outstanding Graduate Award. Currently working at the Rocky Mountain Labs, National Institute of Allergy and Infectious Diseases
Karen Ortiz-Cruz	Graduated	Graduated with honors. Currently working as a summer student in the NCI. Wants to attend graduate school at NCI.
Meagan Govig	Graduated	Cum laude, Beta Beta Beta, one of two Biology Majors (out of over 100) accepted into the Blue Key Society, a leadership society. Will attend U. Iowa Dental School starting Fall 2002.
Janelle Kime	Rising senior	Summer undergraduate research fellow at Mayo Clinic, Dept. Immunology. Awarded Barry M. Goldwater scholarship.
Carmen Garst	Rising senior	Beta Beta Beta member. Currently a summer undergraduate research fellow at Iowa State University.

III. Key Research Accomplishments

- Kime et al used cDNA microarrays to investigate how metastasis might be influenced by an unusual tandem repeat in MUC1, a tumor-associated antigen in breast cancer and other tumor types. By comparing gene expression from cell lines with or without expression of the MUC1 tandem repeat, they found that thrombospondin-1 was maintained the greatest level of differential gene expression.
- Garst et al developed retroviral vectors expressing human telomerase RNA. Telomerase is an enzyme that is crucial in controlling the lifespan of human cells. Many tumors, including breast tumors, express telomerase inappropriately, allowing those cells to become immortal. The project by Garst et al was to establish retroviral vectors that can be used as tools to immortalize many different types of cells in culture, with the goal of better understanding the role of telomerase in cancer.
- West et al investigated new reagents for generating gene knockouts of potential breast cancer tumor suppressors. The purpose of this study was to improve the cre-lox gene knockout technology such that the cre recombinase expression was controllable, using a chimeric cre coding region under the control of a variant progesterone receptor. The receptor-cre chimera would therefore inducible by addition of RU486. They found that cre activity in mouse embryonic fibroblasts was, to a large extent, well controlled by RU486. The laboratory will next use this new technology to knockout Tumor Susceptibility Gene 101 (TSG101), a putative breast cancer tumor suppressor.
- Ortiz et al developed new transgenic mice heterozygous for a breast cancer tumor promoter called KSR. KSR, inaccurately named as kinase suppressor of ras, actually helps promote ras-mediated tumors in mice and ras-mediated transformation in cell culture. To better understand the role of KSR in breast cancer promotion, Ortiz and coworkers are crossing the heterozygous mutation into different mouse strains. The major purpose of this work will be to identify potential interacting genes that work with KSR in breast cancer promotion.

- Govig et al studied how body weight is genetically controlled in ACI and Copenhagen rat strains, two animal systems where estrogen-mediated breast cancer susceptibility is under genetic control. They investigated the association between genetically determined body mass and genetically confered susceptibility to estrogen-induced breast cancer. Two weight controlling loci, Grr1 and Grr2 were mapped to chromosomes 8 and 10, whereas previously mapped mammary cancer susceptibility loci reside on chromosomes 5 and 18.

IV. Reportable Outcomes

1. Each student presented her research at the UNMC Poster Day, August 2, 2001.

2. One abstract on this work has been published.
   Functional analysis of MUC1 domains using cDNA and oligonucleotide microarrays.
   Gawron, A. J., Kelly, D., **Kime, J.**, Kohlgraf, K., Hollingsworth, M. A.
   American Association for Cancer Research, April 6-10, 2002. San Francisco, CA. **32**:636, 2002.

3. A second abstract was presented by the student at the 2001 SACNAS meeting.
   Generation of $KSR^{-/-}$ Mice in Multiple Genetic Backgrounds.
   Karen L. Ortiz, Robert L. Kortum and Robert E. Lewis.
   Society for Advancement of Chicanos and Native Americans in Science. 2001.

4. Four of the students have continued on in biomedical research. The fifth, Meagan Govig, will be starting denatl school in the fall.

- Janelle Kime is a Summer Undergraduate Research Fellow at the Mayo Clinic (Rochester, MN), Department of Immunology. Janelle was awarded a Barry M. Goldwater Scholarship for her summer internship.
- Sadie West is currently working at the Rocky Mountain Labs, National Institute of Allergy and Infectious Diseases, identifying the function of chlamydial inclusion proteins.
- Karen Ortiz Cruz is doing a summer internship at the National Cancer Institute.
- Carmen Garst is a summer undergraduate research fellow at Iowa State University.

V. Conclusions

In the first year of the BCTP-SU, five outstanding students were recruited to the Eppley Institute, where they performed research on breast cancer and other types of cancer. The students were highly motivated and successful in their projects. Three students helped develop new tools for breast cancer research (microarrays, improved gene knockout reagents, telomerase vectors) and the other two students evaluated genes implicated in breast cancer and growth control. All five students have indicated their intention to continue on in research and/or medical fields, consistent with the goals of the BCTP-SU. Two have continued on in research tracts, and one other will be starting dental school. The other two students are rising seniors in college.

Recommendations for current and future years of the BCTP-SU are:

1. Continue to identify, recruit, and train outstanding undergraduate students.
2. Continue to focus on breast cancer research training for these students.
3. Continue to recruit women and other under-represented minorities.
4. Continue to maintain tracking of previous students.

Utilization of cDNA Microarrays to Investigate the Role of the MUC1 Tandem Repeat in Gene Expression.

Janelle A. Kime, Andrew J. Gawron, David L. Kelly, M.A. Hollingsworth

MUC1 is a membrane-associated glycoprotein that is differentially glycosylated and over-expressed in tumor cells. It contains an extracellular tandem repeat sequence of variable length which potentially plays a role in metastasis. In order to investigate the role of the tandem repeat in tumor metastasis, we chose to investigate the gene expression profile of two pancreatic cancer cell lines expressing MUC1 both with and without the tandem repeat using cDNA microarrays. RNA was isolated from two S2-013 cell lines and fluorescent dyes were incorporated into cDNA via reverse transcription. Labeled samples were hybridized to the microarray and scanned images generated ratios of gene expression intensity. Genes with a two-fold difference or greater differential were determined to be up-regulated in the respective cell line. Thrombospondin-1 maintained the greatest level of differential gene expression in the MUC1F tandem repeat deleted cell line, which was confirmed by Northern Blot Analysis. Based upon the up-regulation of Thrombospondin-1 with tandem repeat deletion,we propose that the expression of MUC1 full length may block the binding of Thrombospondin-1 to its receptors, thus implying that the deletion of the tandem repeat allows Thrombospondin-1 access to receptors on lymphatic cells. Thrombospondin expression could also serve to compensate for the loss of the tandem repeat, which causes metastasis to a different organ system by a different mechanism. Alternatively, tandem repeat deletion may alter signal transduction of the cytoplasmic tail of MUC1, causing Thrombospondin-1 to be up-regulated.

The Design and Characterization of Retroviral Vectors Expressing Human Telomerase RNA.

Carmen M. Garst, Amy Farrel, Michel M. Ouellette

The enzyme telomerase plays a crucial role in controlling the lifespan of human cells. The enzyme is responsible for the synthesis and maintenance of telomeres, essential structures that cap and protect the ends of chromosomes. Most somatic human cells lack the activity, are unable to maintain their telomeres and enter senescence after a finite lifespan. The enzyme is present in immortal cells (germline lineage, cancer cells) and in cells that display longer lifespan (lymphocytes, rare stem cells of the blood, skin and digestive system). The enzyme contains two essential subunits: the protein hTERT (human Telomerase Reverse Transcriptase) and the small nuclear RNA hTR (human Telomerase RNA). The goal of the present study is to test retroviral vectors that we have designed and created for the transfer and stable expression of hTR into human cells. Retroviruses are ideal vehicles for transferring foreign genes to mammalian ! cells. First, they can transfer genetic material with great efficiency to a large spectrum of cell types. Second, the transferred genes are rapidly integrated in the genome of the host, allowing stable expression of the transferred genes. Such vectors would allow studies of the structure/function relationship of hTR to be performed in the context of living cells with the possibility of examining the long term effects on hTR on cellular lifespan.

Generation of $KSR^{-/-}$ Mice in Multiple Genetic Backgrounds.

Karen L. Ortiz, Robert L. Kortum and Robert E. Lewis.

Kinase suppressor of Ras (KSR) was identified previously as a loss-of-function allele that reverts the rough eye phenotype of activated Ras in *Drosophila* and the multiple vulval phenotype of activated Ras in *Caenorhabditis elegans*. $KSR^{-/-}$ mice appear fertile and otherwise normal on a DBA/1J genetic background, however, signaling and tumorigenesis via activated Ras are impaired. Other genetic models have shown that genetic background affects phenotype. Therefore, we are producing $KSR^{-/-}$ mice in both FVB/NJ and C57B1/6J backgrounds. Traditional backcrossing requires 12 generations, or 3-4 years, to produce a congenic mouse strain. Speed congenics, however, can produce congenic strains in 3-4 backcross generations by screening each gene with markers to eliminate the maximal amount of contaminating genome. We have begun this process, thus far eliminating 71% of the contaminating genome for the FVB/NJ backcross and 60 % of the contaminating genome for the C57B1/6J backcross. Once complete, we will evaluate general development, cell signaling through the MAP kinase cascade, and tumorigenesis by activated Ras in the absence of KSR in each of these genetic backgrounds.

In Vitro Gene Deletion Using a Ligand-Inducible Cre Recombinase Expressed by a Newly-Developed Retroviral Vector.

Sadie West, Andrea Krempler, Kay-Uwe Wagner.

Aim and Purpose: The aim is to set up an *in vitro* system that uses a retroviral vector to delete any gene of interest in an inducible manner (temporal knockout). We need to temporarily control the activity of the Cre recombinase since prolonged exposure of cells to Cre results in genomic instability *in vitro*. In some cases, the excision of the gene of interest results in growth inhibition and/or cell death. By attaching Cre to a mutated ligand-binding domain of a progesterone receptor (PR2), unwanted background recombinase activity in the absence of the synthetic ligand RU486 is reduced, and RU486 can be utilized to activate the recombination at a desired time point. Therefore, the function of the gene of interest can be studied without premature mortality. *Conclusions:* We have generated a retrovirus containing an RU486-inducible Cre recombinase (Cre-PR2) that can be used for temporally regulated gene knockouts *in vitro*. The inducebility of the CrePR2 fusion protein by RU486 was tested using a loxP reporter assay in mouse embryonic fibroblasts that stably expresses Cre-PR2 in addition to the puromycin selection marker. The activity of the Cre recombinase is greatly induced by RU486. Some Cre recombinase activity was observed also in the absence of RU486. This leakiness might be a problem for future experiments where a very tight regulation of the Cre activity is critical. In future experiments we will utilize the newly developed inducible system to delete the Tumor Susceptibility Gene 101 (TSG101) gene in mouse embryonic fibroblasts and other primary cell lines. The leakiness of the system is not critical for these proposed studies since cells that do not tightly control the activity of Cre and therefore lack TSG101 do not divide and will die (negative selection).

Genetic Control of Growth in the ACI and Copenhagen Rat Srains

Meagan Govig, Tracy Strecker, Mac McLaughlin, Martin Tochacek, Karen Pennington, and James Shull

Body weight is a significant factor in determining an increased risk for illnesses such as heart disease, diabetes, and cancer. A better understanding of this relationship between body weight and susceptibility to disease may be gained by studying genetic control of growth. Crosses between the genetically related ACI and COP (Copenhagen) rat strains were studied in order to map possible regions of the genome responsible for modifying growth in the rat. Utilizing body weight data from the F2 progeny of ACIxCOP intercrosses, two quantitative trait loci (QTLs), Grr1 and Grr2, were mapped to chromosomes 8 and 10, respectively. Continued identification and study of genes within these loci may lend insight into a more complete understanding of genetic modification of growth.